



**Whole Effluent Toxicity Test Report:
Washington Beef LLC.**

December 2012

Report date: December 26, 2012

Submitted to:

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1.0 INTRODUCTION

A whole effluent toxicity test was conducted using effluent samples collected from the Washington Beef LLC wastewater treatment plants in December 2012. A chronic bioassay was conducted using the test organism *Ceriodaphnia dubia* (*Ceriodaphnia*). Testing was performed at Rainier Environmental Laboratory located in Tacoma, Washington.

2.0 METHODS

2.1 Sample Collection and Transport

Effluent samples were collected into 4-liter (L) LDPE cubitainers by Washington Beef personnel. The samples were packed in coolers containing ice and shipped to Rainier Environmental by overnight delivery service. Appropriate chain-of-custody procedures were employed during collection and transport (Appendix D).

2.2 Sample Receipt

Upon arrival at the laboratory, coolers were opened, samples inspected, and the contents verified against information provided on the chain-of-custody forms. Receipt temperature was measured and recorded on the chain-of-custody form. The standard water quality parameters were measured and recorded on sample check-in sheets (Appendix B). Samples were stored at 4°C in the dark until used for testing.

2.3 Test Methods

A chronic toxicity test was conducted according to procedures presented by USEPA (2002). The methods are summarized in Table 1. The procedure involved a 7-day static-renewal exposure to the effluent. The endpoints from these tests were *Ceriodaphnia* survival at the end of exposure and reproduction at test termination or production of 3 broods, whichever occurred first. Termination of the test occurred when at least 60 percent of surviving control females produced 3 broods. The test was ended on Day 7.

Table 1. Summary of methods for the 7-day *Ceriodaphnia* survival and reproduction test.

Test initiation date and time	12/11/12; 1500h
Test termination date and time	12/18/12; 1415h
Test organism	<i>Ceriodaphnia dubia</i>
Test organism source	In-house cultures
Test organism age	< 24 hours
Test duration	7 days; Test terminated when 60% of controls reached 3 broods
Feeding	1:1 mixture YTC:algal suspension daily
Test chamber; test solution volume	30 mL plastic cup; 15 mL
Test temperature	25 ± 1°C
Dilution water	Diluted mineral water
Test concentrations (% sample)	100, 50, 25, 12.5, 6.25, laboratory control
Number of organisms/chamber	1
Number of replicates	10
Photoperiod	16 hours light/8 hours dark
Aeration	None
Test protocol	EPA-821-R-02-013
Test acceptability criteria for controls	≥ 80% survival; ≥ 15 neonates per surviving adult
Reference toxicant	Sodium chloride

3.0 RESULTS AND DISCUSSION

Details of standard water quality measurements conducted upon receipt of samples are provided in Table 2.

Table 2. Final Effluent sample information.

Parameter	Final Effluent		
Rainier Log-in No.	12-053	12-054	12-055
Collection date and time	12/10/2012; 0725h	12/12/2012; 0740h	12/14/2012; 0718h
Receipt date and time	12/11/2012; 1400h	12/13/2012; 0930h	12/15/2012; 0940h
Receipt temperature (°C)	0.3	0.3	0.5
Dissolved oxygen (mg/L)	9.1	8.3	7.8
pH	7.78	7.76	8.00
Conductivity (µS/cm)	4260	4330	4280
Salinity (ppt)	2.1	2.0	2.1
Hardness (mg/L CaCO ₃)	68	68	72
Alkalinity (mg/L CaCO ₃)	132	140	128
Total Chlorine (mg/L) ^a	<0.03	<0.03	<0.03
Total Ammonia (mg/L) ^b	<1.0	<1.0	<1.0

^{a,b} See reference below

Note: Total chlorine and ammonia values are measured by Rainier Environmental to provide additional information in support of the bioassay test procedures. They are not intended to be interpreted as exact values, particularly near the detection limits where interferences are most likely to become apparent.

^a Total chlorine is measured using a Hach DR/2000 spectrophotometer and colorimetric DPD Total Chlorine Reagent. Under optimum conditions, the method has a range of 0.03 to 2.0 mg/L \pm 0.01 mg/L total chlorine. Compounds in the sample that interfere with chlorine detection include bromine, manganese, chromium, ozone, and peroxides. Additional interferences include extreme pH values and high alkalinity (greater than 300 mg/L Ca CO₃).

^b Total ammonia is measured using a Hach DR/2000 spectrophotometer following the salicylate method which uses AmVer Diluent Reagent Test 'N' Tube kits. Under optimum conditions, the method has a range of 0.4 to 50.0 \pm 0.1 mg/L NH₃-N. High sample turbidity will give erroneously high values. Additional interferences to the method include extreme pH and high concentrations of magnesium, iron, nitrite, nitrate, or sulfate.

Results for the toxicity tests are summarized in Table 3. Individual statistical summaries for the test and copies of the laboratory bench sheets are provided in the Appendices A-D.

The NOEC (concentration at which no effect on the organisms is detected) was 100 percent sample for survival and 50 percent for reproduction. The associated chronic toxicity unit (TUC; 100 percent sample divided by the NOEC) was 1 for survival and 2 for reproduction.

Table 3. Summary of toxicity test results.

Sample	Endpoint	NOEC (% effluent)	Chronic Toxicity Unit (TUC) ^a
Final Effluent	Survival	100	1.0
	7-day Reproduction	50	2.0

^a Chronic toxicity unit (TUC = 100 \div NOEC)

4.0 QA/QC

Samples were received in good condition and within the temperature range specified by EPA (2002). The toxicity tests met all acceptability criteria for performance of control organisms. There were no deviations from protocol and water quality parameters remained within the ranges specified in the corresponding test methods throughout the tests.

Results for the most recent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 4 and Appendix C. The coefficients of variation (CV) for the endpoints are also shown in the table. The results for the reference toxicant test fell within the acceptable range of mean \pm two standard deviations of historical test results indicating that the test organisms were of an appropriate degree of sensitivity.

Table 4. Reference toxicant test results.

Species	Endpoint	Date initiated	LC ₅₀ /EC ₅₀	Acceptable Range	CV (%)
<i>Ceriodaphnia</i>	7d survival	12/11/2012	2.46 g/L NaCl	1.26 – 2.72 g/L	21.2
	7d reproduction	12/11/2012	1.44 g/L NaCl	1.07 - 1.52 g/L	9.2

REFERENCES

Tidepool Scientific Software. 2000-2010. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.0.8.

USEPA. 2002. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, Fourth Edition. EPA-821-R-02-013. pp. 141-196.

Appendix A
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 22 Dec-12 10:57 (p 1 of 2)
 Test Code: 1212-016 | 21-3673-3775

Ceriodaphnia 7-d Survival and Reproduction Test					Rainier Environmental Laboratory	
Batch ID:	15-9309-1244	Test Type:	Reproduction-Survival (7d)		Analyst:	Eric Tollefson
Start Date:	11 Dec-12 15:00	Protocol:	EPA/821/R-02-013 (2002)		Diluent:	Perrier Water
Ending Date:	18 Dec-12 14:15	Species:	Ceriodaphnia dubia		Brine:	
Duration:	6d 23h	Source:	In-House Culture		Age:	<24h
Sample ID:	02-8942-5277	Code:	12-053		Client:	Washington Beef
Sample Date:	10 Dec-12 07:25	Material:	POTW Effluent		Project:	
Receive Date:	11 Dec-12 14:00	Source:	Washington Beef (WA0050202)			
Sample Age:	32h (0.3 °C)	Station:				

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-6632-5015	7d Survival Rate	100	>100	NA	NA	1	Fisher Exact/Bonferroni-Holm Test
12-2901-2093	Reproduction	50	100	70.71	33.0%	2	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
02-9665-8376	Reproduction	IC5	1.495	0.4347	51.22	66.91	Linear Interpolation (ICPIN)
		IC10	5.223	1.058	53.84	19.15	
		IC15	36.93	1.953	56.58	2.708	
		IC20	51.13	3.237	59.77	1.956	
		IC25	54.52	5.079	63.2	1.834	
		IC40	66.08	52.25	74.78	1.513	
		IC50	75.1	62.87	84.12	1.332	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
16-6632-5015	7d Survival Rate	Control Resp	0.9	0.8 - NL	Yes	Passes Acceptability Criteria
02-9665-8376	Reproduction	Control Resp	32.3	15 - NL	Yes	Passes Acceptability Criteria
12-2901-2093	Reproduction	Control Resp	32.3	15 - NL	Yes	Passes Acceptability Criteria
12-2901-2093	Reproduction	PMSD	0.3299	0.13 - 0.47	Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	0.9	0.7819	1	0	1	0.1	0.3162	35.14%	0.0%
6.25		10	0.9	0.7819	1	0	1	0.1	0.3162	35.14%	0.0%
12.5		10	1	1	1	1	1	0	0	0.0%	-11.11%
25		10	1	1	1	1	1	0	0	0.0%	-11.11%
50		10	1	1	1	1	1	0	0	0.0%	-11.11%
100		10	1	1	1	1	1	0	0	0.0%	-11.11%

Reproduction Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	32.3	27.98	36.62	5	44	3.658	11.57	35.81%	0.0%
6.25		10	27.4	22.36	32.44	0	46	4.272	13.51	49.3%	15.17%
12.5		10	27.9	24.32	31.48	14	46	3.035	9.597	34.4%	13.62%
25		10	31.1	26.42	35.78	6	46	3.965	12.54	40.32%	3.72%
50		10	26.4	23.84	28.96	15	37	2.172	6.867	26.01%	18.27%
100		10	8.9	6.615	11.18	0	17	1.935	6.118	68.74%	72.45%

CETIS Summary ReportReport Date: 22 Dec-12 10:57 (p 2 of 2)
Test Code: 1212-016 | 21-3673-3775**Ceriodaphnia 7-d Survival and Reproduction Test****Rainier Environmental Laboratory****7d Survival Rate Detail**

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	0	1
6.25		1	1	1	1	1	1	1	0	1	1
12.5		1	1	1	1	1	1	1	1	1	1
25		1	1	1	1	1	1	1	1	1	1
50		1	1	1	1	1	1	1	1	1	1
100		1	1	1	1	1	1	1	1	1	1

Reproduction Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	38	33	34	21	39	44	41	38	5	30
6.25		27	32	30	44	46	22	26	0	33	14
12.5		32	27	27	31	22	18	46	23	39	14
25		38	32	38	40	23	35	46	6	38	15
50		37	31	35	27	26	20	22	22	29	15
100		16	3	14	17	2	7	0	13	11	6

7d Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1
6.25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1	1/1
12.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
50		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
100		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

Rainier Environmental
Washington Laboratory

Client: Washington Beach
Sample ID: Final Effluent outfall 002
Test No: 1212-016
Log-In#: 12-053 12-054

Initial and Final Chemistries
Seven Day Chronic Freshwater Bioassay
Start Date & Time: 12/11/12 1500
Stop Date & Time: 12/18/12 1415
Test Species: Ceriodaphnia dubia
12-055

Conc. or % CON	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.83	7.93	7.75	7.85	7.95	7.73	7.63	7.67	7.71	7.81	7.73	7.90	7.85	7.95
DO (mg/l)	8.2	8.1	8.0	8.2	8.0	8.1	7.7	8.1	7.8	8.0	7.7	8.1	7.8	8.2
Cond. (μmhos-cm)	277	283	292	301	295	302	292	301	295	302	301	308	292	297
Temperature (°C)	25.2	25.2	25.5	25.3	25.0	25.1	24.6	25.1	24.4	25.4	25.1	25.9	24.9	25.3
6.25	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.66	7.93	7.77	7.91	7.94	7.73	7.54	7.71	7.71	7.85	7.74	7.86	7.82	7.81
DO (mg/l)	8.2	8.2	8.1	8.2	8.0	8.2	7.6	7.8	8.0	7.9	7.8	8.0	7.9	8.2
Cond. (μmhos-cm)	543	547	553	555	555	555	571	575	565	567	545	547	551	561
Temperature (°C)	25.2	25.3	25.2	25.4	24.7	25.2	24.7	25.4	24.7	25.4	24.8	25.5	25.0	25.4
12.5	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.62	7.95	7.81	7.87	7.94	7.77	7.57	7.72	7.76	7.81	7.79	7.89	7.81	7.93
DO (mg/l)	8.2	8.2	8.2	8.1	8.1	8.2	7.8	8.1	7.9	8.0	7.9	8.2	7.9	8.1
Cond. (μmhos-cm)	794	805	793	802	800	807	849	855	847	851	827	835	837	843
Temperature (°C)	25.2	25.3	25.0	25.4	24.7	25.2	24.7	25.4	24.8	25.5	24.8	25.4	25.0	25.4
25	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.63	8.01	7.85	7.89	7.97	7.75	7.62	7.75	7.75	7.83	7.81	7.87	7.81	7.99
DO (mg/l)	8.1	8.2	8.2	8.1	8.1	8.1	8.0	8.0	7.9	8.1	8.0	8.2	8.0	8.1
Cond. (μmhos-cm)	1388	1396	1292	1305	1291	1392	1374	1392	1325	1325	1305	1312	1302	1315
Temperature (°C)	25.2	25.3	25.0	25.4	25.1	25.2	24.9	25.4	24.8	25.5	25.2	25.4	24.8	25.4
50	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.68	8.05	7.91	7.93	7.96	7.71	7.78	7.82	7.72	7.84	7.83	7.81	7.85	8.01
DO (mg/l)	8.1	8.1	8.0	8.0	8.1	8.2	8.0	8.1	8.1	8.2	7.8	8.0	8.0	8.2
Cond. (μmhos-cm)	2307	2321	2278	2208	2305	2313	2382	2395	2402	2413	2357	2362	2362	2371
Temperature (°C)	25.1	25.3	24.9	25.4	25.3	25.2	25.1	25.4	24.8	25.5	25.4	25.4	24.7	25.4
100	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.73	8.11	7.93	7.98	7.91	7.82	7.96	7.84	7.81	7.85	7.90	7.78	7.87	7.98
DO (mg/l)	8.0	8.2	7.8	8.2	8.1	8.2	7.9	8.1	8.0	8.1	7.7	7.8	7.6	8.1
Cond. (μmhos-cm)	4270	4300	4230	4250	4250	4270	4410	4440	4420	4470	4370	4400	4290	4380
Temperature (°C)	25.0	25.3	24.7	25.4	25.5	25.2	25.4	25.5	24.9	25.5	25.6	25.4	24.5	25.4
Tech. Initials	gt													

Dilution Water Batch #: 8.2 006
Test Chamber: VWR

QA Check: gt

Sample Description:
Animal Source: In House Culture
Comments:

Date Received: _____ Date of Hatch: _____

Client/Sample ID:

Washington Beef

Ceriodaphnia 7-Day Chronic Survival and Reproduction

Start Date and Time:

12/1/12 1500

Stop Date and Time:

12/8/12 1415

Rep	Conc.	Cont	Daily Reproduction							Day 6 Total Brood
			1	2	3	4	5	6	7	
1	CON	5	1	1	1	5	1	3	13	39
2	1a	1a	1	1	1	4	1	7	22	33
3	39	1	1	1	1	3	11	1	14	34
4	32	1	1	1	1	1	6	15	21	31
5	38	1	1	1	1	6	1	12	21	31
6	14	1	1	1	1	4	1	15	19	44
7	52	1	1	1	1	4	1	15	19	44
8	45	1	1	1	5	1	11	1	22	19
9	42	1	1	1	5	1	5	1	16	38
10	7	1	1	1	5	1	5	1	5	5
Analyst	4	4	3t	3t	3t	3t	3t	3t	3t	30
Time	1500	1345	1315	1000	1045	945	1145	1455		
Selen #	007	007	007	007	007	007	007	007		
Rep	Conc.	Cont	1	2	3	4	5	6	7	8
Total										

Rep	Conc.	Cont	Daily Reproduction							Day 6 Total Brood
			1	2	3	4	5	6	7	
1	6.35	3	1	1	1	3	1	6	9	37
2	50	1	1	1	6	2	1	6	11	10
3	15	1	1	1	3	1	6	1	10	32
4	21	1	1	1	1	9	1	14	21	30
5	10	1	1	1	1	7	1	15	23	44
6	23	1	1	1	2	1	7	15	24	46
7	55	1	1	1	3	6	1	17	20	47
8	46	1	1	1	3	6	1	17	20	47
9	54	1	1	1	4	1	14	1	15	36
10	11	1	1	1	4	2	1	4	8	15

Rep	Conc.	Cont	Daily Reproduction							Day 6 Total Brood
			1	2	3	4	5	6	7	
1	1aS	43	1	1	1	7	1	11	14	16
2	37	1	1	1	1	6	1	10	11	27
3	13	1	1	1	1	3	1	14	17	37
4	59	1	1	1	1	1	13	1	13	31
5	44	1	1	1	1	1	10	1	13	22
6	8	1	1	1	1	5	1	13	5	19
7	24	1	1	1	3	10	1	23	23	46
8	36	1	1	3	1	12	1	8	8	20
9	27	1	1	4	1	13	1	22	17	39
10	33	1	1	1	3	1	4	7	14	31

Comments: _____ X=mortality

OA _____

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Appendix B
Sample Check-In Sheets

Rainier Environmental
5013 Pacific Hwy East, Ste. 20
Tacoma, WA 98424

Sample Check-In Information

Client: Washington Beef

Tests Performed: Cause Chronic
Test ID No(s): 1212-016

Sample Description:
Light Brown

Sample ID:	<u>WET</u>	<u>Effluent</u>	<u>Filtrate</u>	
Login No. (10-xxxx):	<u>12-053</u>	<u>12-054</u>	<u>12-055</u>	
Sample Collection Date & Time:	<u>12/10/12 7:25</u>	<u>12/10/12 7:40</u>	<u>12/10/12 7:40</u>	
Sample Receipt Date & Time:	<u>12/11/12 14:00</u>	<u>12/13/12 9:30</u>	<u>12/15/12 7:40</u>	
Check-in Temperature (°C)	<u>0.3</u>	<u>0.3</u>	<u>0.5</u>	
Temperature OK?	<input checked="" type="radio"/> N			
DO (mg/L)	<u>9.1</u>	<u>8.3</u>	<u>7.8</u>	
pH (units)	<u>7.78</u>	<u>7.76</u>	<u>8.00</u>	
Conductivity ($\mu\text{S}/\text{cm}$)	<u>4260</u>	<u>4330</u>	<u>4280</u>	
Salinity (ppt)	<u>2.1</u>	<u>2.0</u>	<u>2.1</u>	
Tit. Vol / Sam. Vol. / Alkalinity (mg/L)*	<u>3.3 / 2.5 / 132</u>	<u>3.5 / 2.5 / 140</u>	<u>3.2 / 2.5 / 128</u>	<u>1 / 1</u>
Tit. Vol. / Sam. Vol. / Hardness (mg/L)* ^a	<u>1.7 / 2.5 / 68</u>	<u>1.7 / 2.5 / 68</u>	<u>1.8 / 2.5 / 72</u>	<u>1 / 1</u>
Total Chlorine (mg/L)	<u><0.03</u>	<u><0.03</u>	<u><0.03</u>	
Total Ammonia (mg/L)	<u><1.0</u>	<u><1.0</u>	<u><1.0</u>	
Technician Initials	<u>BT</u>	<u>BT</u>	<u>BT</u>	

* = mg/L as CaCO₃, ^a = Measured for freshwater samples only, NA = Not Applicable.

NM = Not Measured

Freshwater Tests:

Control/Dilution Water Source: test type: CD-C 8:2 (DMWW) MHW Other: _____ Alkalinity: 64 Hardness: 84

Control/Dilution Water Source: test type: 8:2 (DMWW) MHW Other: _____ Alkalinity: _____ Hardness: _____

Additional Control? Y N = _____

Alkalinity: _____ Hardness: _____

Aeration? Y N

Length of Time: _____
Final DO: _____
Final pH: _____

Hardness Adjustment? Y N
If adjusted, please see worksheet
for details.

Marine Tests:

Control/Dilution Water Source: test type: ART SW NAT SW Alkalinity: _____ Salinity: _____

Control/Dilution Water Source: test type: ART SW NAT SW Alkalinity: _____ Salinity: _____

Additional Control? Y N = _____

Alkalinity: _____ Salinity: _____

Sample Salted w/ artificial salt? Y N If yes, what ppt? _____ test type: _____

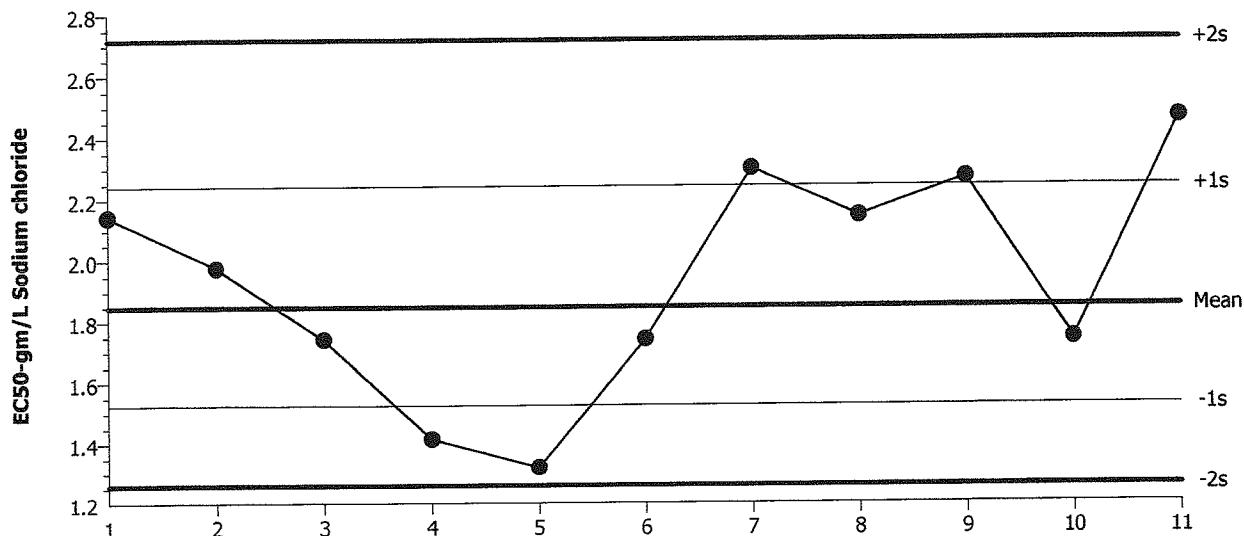
Sample salted w/brine? Y N If yes, what ppt? _____ test type: _____

Comments: Temperature for grab sample must be 0-20°C if received within 1 hour of collection time, 0-12°C if effluent received within 4 hours of collection time, and 0-6°C for all other samples.

QC Check: BT

Appendix C
Reference Toxicant Test

Ceriodaphnia 7-d Survival and Reproduction Test			Rainier Environmental Laboratory		
Test Type:	Reproduction-Survival (7d)	Organism:	Ceriodaphnia dubia (Water Flea)	Material:	Sodium chloride
Protocol:	EPA/821/R-02-013 (2002)	Endpoint:	7d Survival Rate	Source:	Reference Toxicant-REF

Ceriodaphnia 7-d Survival and Reproduction Test

Mean: 1.849 Count: 10 -1s Warning Limit: 1.525 -2s Action Limit: 1.258
Sigma: NA CV: 21.20% +1s Warning Limit: 2.241 +2s Action Limit: 2.716

Quality Control Data

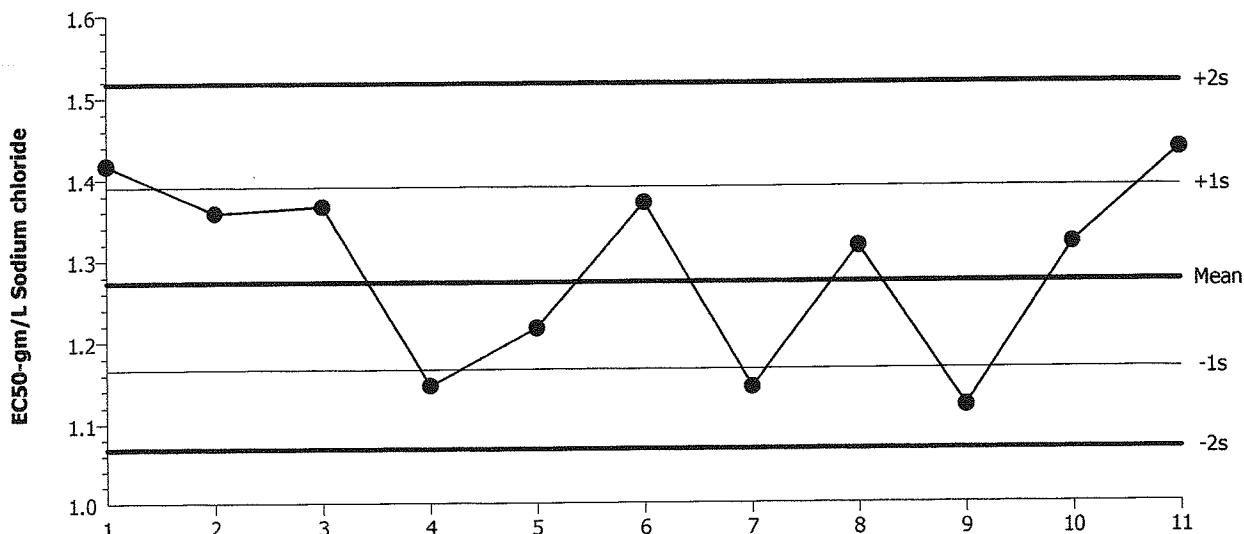
Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Nov	10	2.144	0.295	0.7696			18-1646-5148	10-8016-4521
2			18	1.977	0.1285	0.3492			11-7820-4865	00-4433-4101
3			19	1.741	-0.1074	-0.3112			08-1813-0818	01-3437-6850
4			25	1.414	-0.4343	-1.392	(-)		12-3676-9409	20-8924-6879
5		Dec	2	1.32	-0.529	-1.752	(-)		03-1806-9758	03-8081-7233
6	2012	Mar	20	1.741	-0.1074	-0.3112			04-4181-4802	06-8447-2630
7		Jun	26	2.297	0.4488	1.13	(+)		18-3335-1100	10-1106-6325
8		Jul	17	2.144	0.295	0.7696			02-9547-9197	16-5989-6607
9		Sep	18	2.267	0.4181	1.06	(+)		20-3257-9401	11-6459-7205
10		Oct	30	1.741	-0.1074	-0.3112			14-7011-9138	01-0378-8759
11		Dec	11	2.462	0.6137	1.49	(+)		18-7111-4230	17-4716-3730

Ceriodaphnia 7-d Survival and Reproduction Test

Rainier Environmental Laboratory

Test Type: Reproduction-Survival (7d)
Protocol: EPA/821/R-02-013 (2002)Organism: Ceriodaphnia dubia (Water Flea)
Endpoint: ReproductionMaterial: Sodium chloride
Source: Reference Toxicant-REF

Ceriodaphnia 7-d Survival and Reproduction Test



Mean: 1.273 Count: 10 -1s Warning Limit: 1.166 -2s Action Limit: 1.068
Sigma: NA CV: 9.17% +1s Warning Limit: 1.39 +2s Action Limit: 1.517

Quality Control Data

Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Nov	10	1.417	0.1444	1.224	(+)		18-1646-5148	02-8001-5569
2			18	1.358	0.08499	0.7367			11-7820-4865	14-3007-5816
3			19	1.366	0.09286	0.8026			08-1813-0818	00-3657-8188
4			25	1.146	-0.1269	-1.197	(-)		12-3676-9409	18-2334-7627
5		Dec	2	1.216	-0.05655	-0.5179			03-1806-9758	11-1567-8111
6	2012	Mar	20	1.37	0.09722	0.8388			04-4181-4802	14-9819-7042
7		Jun	26	1.144	-0.1292	-1.219	(-)		18-3335-1100	06-3063-9294
8		Jul	17	1.316	0.04323	0.3806			02-9547-9197	03-2978-8518
9		Sep	18	1.121	-0.1524	-1.453	(-)		20-3257-9401	21-2717-5233
10		Oct	30	1.319	0.04643	0.4083			14-7011-9138	13-0927-4963
11		Dec	11	1.435	0.1622	1.367	(+)		18-7111-4230	00-5310-2281

CETIS Summary Report

Report Date: 22 Dec-12 10:27 (p 1 of 2)
 Test Code: RT121112CD | 18-7111-4230

Ceriodaphnia 7-d Survival and Reproduction Test				Rainier Environmental Laboratory	
Batch ID:	09-1404-9680	Test Type:	Reproduction-Survival (7d)	Analyst:	Eric Tollefson
Start Date:	11 Dec-12 11:45	Protocol:	EPA/821/R-02-013 (2002)	Diluent:	Mod-Hard Synthetic Water
Ending Date:	18 Dec-12 12:45	Species:	Ceriodaphnia dubia	Brine:	
Duration:	7d 1h	Source:	In-House Culture	Age:	<24h
Sample ID:	15-1059-9868	Code:	RT121112CD	Client:	Internal Lab
Sample Date:	11 Dec-12 11:45	Material:	Sodium chloride	Project:	
Receive Date:	11 Dec-12 11:45	Source:	Reference Toxicant		
Sample Age:	NA	Station:	In House		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-9789-2919	7d Survival Rate	2	4	2.828	NA		Fisher Exact/Bonferroni-Holm Test
11-3856-2034	Reproduction	1	2	1.414	18.9%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	gm/L	95% LCL	95% UCL	TU	Method
17-4716-3730	7d Survival Rate	LC50	2.462	2.066	2.934		Spearman-Kärber
00-5310-2281	Reproduction	IC5	0.6127	0.3532	0.7914		Linear Interpolation (ICPIN)
		IC10	0.7339	0.5202	1.024		
		IC15	0.8643	0.6391	1.082		
		IC20	1.002	0.7368	1.143		
		IC25	1.068	0.8215	1.205		
		IC40	1.281	1.077	1.401		
		IC50	1.435	1.266	1.547		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC	Limits	Overlap	Decision
09-9789-2919	7d Survival Rate	Control Resp	1	0.8 - NL		Yes	Passes Acceptability Criteria
17-4716-3730	7d Survival Rate	Control Resp	1	0.8 - NL		Yes	Passes Acceptability Criteria
00-5310-2281	Reproduction	Control Resp	33.2	15 - NL		Yes	Passes Acceptability Criteria
11-3856-2034	Reproduction	Control Resp	33.2	15 - NL		Yes	Passes Acceptability Criteria
11-3856-2034	Reproduction	PMSD	0.1893	0.13 - 0.47		Yes	Passes Acceptability Criteria

7d Survival Rate Summary

C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	1	1	1	1	1	0	0	0.0%	0.0%
0.25		10	1	1	1	1	1	0	0	0.0%	0.0%
0.5		10	1	1	1	1	1	0	0	0.0%	0.0%
1		10	1	1	1	1	1	0	0	0.0%	0.0%
2		10	0.8	0.6426	0.9574	0	1	0.1333	0.4216	52.7%	20.0%
4		10	0	0	0	0	0	0	0		100.0%

Reproduction Summary

C-gm/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	10	33.2	29.82	36.58	14	41	2.859	9.041	27.23%	0.0%
0.25		10	36.9	35.63	38.17	33	43	1.08	3.414	9.25%	-11.14%
0.5		10	36.2	34.22	38.18	24	40	1.679	5.308	14.66%	-9.04%
1		10	28.4	25.6	31.2	15	37	2.372	7.501	26.41%	14.46%
2		10	6.4	4.647	8.153	0	13	1.485	4.695	73.36%	80.72%
4		10	0	0	0	0	0	0	0		100.0%

CETIS Summary Report

Report Date:

22 Dec-12 10:27 (p 2 of 2)

Test Code:

RT121112CD | 18-7111-4230

Ceriodaphnia 7-d Survival and Reproduction Test**Rainier Environmental Laboratory****7d Survival Rate Detail**

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1	1	1	1	1	1	1	1	1	1
0.25		1	1	1	1	1	1	1	1	1	1
0.5		1	1	1	1	1	1	1	1	1	1
1		1	1	1	1	1	1	1	1	1	1
2		1	1	1	1	1	1	0	0	1	1
4		0	0	0	0	0	0	0	0	0	0

Reproduction Detail

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	14	41	32	38	39	41	39	32	35	21
0.25		33	40	34	43	35	35	40	38	38	33
0.5		37	40	39	39	38	38	39	29	39	24
1		27	33	20	37	26	23	37	31	35	15
2		0	13	7	9	10	8	0	0	9	8
4		0	0	0	0	0	0	0	0	0	0

7d Survival Rate Binomials

C-gm/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Dilution Water	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.25		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
0.5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
1		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
2		1/1	1/1	1/1	1/1	1/1	1/1	0/1	0/1	1/1	1/1
4		0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Rainier Environmental
Washington Laboratory

Client: Reference Testant
Sample ID: 4.0 g/L NaCl
Test No: RT 12/11/12 CD
Log-In#:

Initial and Final Chemistries

Seven Day Chronic Freshwater Bioassay

Start Date & Time: 12/11/12 8:45 AM
Stop Date & Time: 12/18/12 12:45
Test Species: Caenidaphnia dubia

Conc. or % <u>CON</u>	Days													
	0		1		2		3		4		5		6	
	init.	final												
pH	7.95	8.00	7.78	7.95	7.92	7.99	7.89	8.01	7.78	7.98	7.87	7.85	7.75	7.91
DO (mg/l)	8.2	8.2	8.0	8.2	7.9	8.1	8.0	8.2	7.8	8.2	8.0	7.8	7.7	8.2
Cond. (umhos-cm)	203	211	195	213	201	212	198	205	202	210	195	212	192	207
Temperature (°C)	25.4	25.2	25.1	25.2	24.7	25.1	24.3	25.2	24.3	25.5	24.5	25.2	24.5	25.5
<u>0.25</u>	Days													
	0	1	2	3	4	5	6	init.	final	init.	final	init.	final	init.
pH	7.92	8.01	7.79	7.92	7.83	7.97	7.90	7.95	7.75	8.02	7.89	7.91	7.73	7.87
DO (mg/l)	7.9	8.2	8.1	8.2	8.0	8.2	8.0	8.2	7.7	8.1	8.2	7.9	7.7	8.2
Cond. (umhos-cm)	724	731	721	742	735	741	719	728	705	720	707	713	707	715
Temperature (°C)	25.4	25.2	25.1	25.4	24.8	25.1	24.5	25.3	24.2	25.5	24.7	25.5	24.4	25.5
<u>0.5</u>	Days													
	0	1	2	3	4	5	6	init.	final	init.	final	init.	final	init.
pH	7.92	7.98	7.81	7.97	7.86	7.95	7.87	7.93	7.76	8.01	7.89	7.93	7.73	7.87
DO (mg/l)	8.0	8.1	8.1	8.2	7.9	8.2	7.9	8.2	7.7	8.1	8.0	8.1	7.5	8.2
Cond. (umhos-cm)	1207	1222	1209	1219	1213	1222	1205	1213	1192	1205	1211	1223	1215	1226
Temperature (°C)	25.4	25.2	25.1	25.4	24.8	25.2	24.5	25.5	24.2	25.5	24.7	25.3	24.4	25.5
<u>1.0</u>	Days													
	0	1	2	3	4	5	6	init.	final	init.	final	init.	final	init.
pH	7.89	7.95	7.85	8.01	7.87	7.92	7.93	7.87	7.75	8.01	7.90	7.93	7.73	7.85
DO (mg/l)	8.2	8.1	8.3	8.2	8.1	8.2	8.1	8.2	7.9	8.0	8.0	8.1	7.7	8.2
Cond. (umhos-cm)	2149	2153	2153	2167	2142	2173	2151	2143	2137	2147	2142	2156	2150	2200
Temperature (°C)	25.4	25.2	25.1	25.5	25.0	25.2	24.5	25.3	24.2	25.5	24.7	25.3	24.4	25.5
<u>2.0</u>	Days													
	0	1	2	3	4	5	6	init.	final	init.	final	init.	final	init.
pH	7.85	7.97	7.87	8.00	7.91	7.96	7.95	7.85	7.75	8.00	7.91	7.89	7.74	7.83
DO (mg/l)	8.2	8.2	8.2	8.2	8.2	8.2	8.1	8.2	7.7	8.2	8.1	8.1	7.7	8.2
Cond. (umhos-cm)	3950	3980	3970	4010	4010	4060	3930	4050	3910	3980	3940	3990	3950	3990
Temperature (°C)	25.4	25.2	25.1	25.5	24.8	25.2	24.5	25.3	24.2	25.5	24.7	25.3	24.4	25.5
<u>4.0 g/L</u>	Days													
	0	1	2	3	4	5	6	init.	final	init.	final	init.	final	init.
pH	7.82	7.98	7.93											
DO (mg/l)	8.1	8.2	8.2											
Cond. (umhos-cm)	7520	7530	7510											
Temperature (°C)	25.4	25.2	25.1											
Tech. Initials	ST													

Dilution Water Batch #: 8:2 008

Test Chamber: VWR

QA Check: ST

Sample Description:

Animal Source: IN HOUSE CULTURE

Date Received:

Date of Hatch:

Comments:

Ceriodaphnia 7-Day Chronic Survival and Reproduction

Start Date and Time:

12/18/12 11:45

Stop Date and Time:

12/18/12 12:45

Client/Sample ID: Rifaini & C.D.
Test Number:

Rep	Conc.	Cont	Daily Reproduction								Day 6 Total	Third Brood
			1	2	3	4	5	6	7	8		
1	CON	35	—	—	6	—	8	—	14	14	14	27
2		54	—	—	6	—	14	21	30	41	14	33
3		42	—	—	5	—	10	—	17	15	32	20
4		18	—	—	1	6	10	—	22	16	39	37
5		10	—	—	3	—	13	—	23	16	39	10
6		3	—	—	4	—	14	—	23	13	41	26
7		15	—	—	4	—	15	—	20	19	39	33
8		9	—	—	6	8	—	18	14	33	16	37
9		41	—	—	5	—	11	—	19	16	35	31
10		40	—	—	7	—	14	—	21	16	35	27
Analyst		X	X	X	X	X	X	X	X	8	9	12
Time		1145	1230	900	830	830	1300	1245				
Selen #		C07	007	007	007	007	007	007	Day 6 Total	Third Brood		
Rep	Conc.	Cont	1	2	3	4	5	6				
1	0.25	16	—	—	1	5	—	6	13	33		
2		22	—	—	1	6	—	12	18	40		
3		11	—	—	1	6	—	10	16	34		
4		31	—	—	4	—	17	—	21	43		
5		6	—	—	3	—	13	—	19	16	35	
6		17	—	—	3	—	9	—	23	12	35	
7		55	—	—	1	6	—	12	18	40		
8		49	—	—	5	11	—	22	16	38		
9		13	—	—	6	14	—	19	20	38		
10		19	—	—	6	—	14	—	21	18	33	

Rep	Conc.	Cont	Daily Reproduction								Day 6 Total	Third Brood
			1	2	3	4	5	6	7	8		
1	0.5	1	—	—	1	3	12	7	22	15	37	0
2		20	—	—	5	—	12	—	23	17	40	0
3		8	—	—	6	—	13	—	20	19	39	0
4		37	—	—	5	—	12	—	22	17	39	4
5		38	—	—	5	—	13	—	20	18	38	5
6		28	—	—	5	—	11	—	22	16	38	6
7		51	—	—	5	2	1	15	22	17	39	5
8		45	—	—	5	6	—	18	11	29	8	34
9		57	—	—	5	1	3	—	21	18	39	9
10		25	—	—	5	4	6	—	14	10	24	10
QA		X	X	X	X	X	X	X	X	0	0	8

Comments: X=mortality

Appendix D
Chain-of-Custody Forms



Washington
5013 Pacific Highway East, Suite 20
Fife
WA 98424
Phone 253.922.8898

Date _____ Page _____ of _____

Sample Collection By: Dennis Perry							ANALYSES REQUIRED
Report to:							Invoice To:
Company	<u>Washington Beef LLC</u>						<u>Washington Beef LLC</u>
Address	<u>201 Springwood Rd</u>						<u>201 Springwood Rd</u>
City/State/Zip	<u>Tupper Lake NY 12985</u>						<u>Tupper Lake NY 12985</u>
Contact	<u>Sherry Burns</u>						<u>Sherry Burns</u>
Phone	<u>(518) 932-6339</u>						<u>(518) 932-6339</u>
Email							
1	WE-T	12/10/12	11:30AM	Cultivator	1	0.3	
2							
3							
4							
5							
6							
7							
8							
9							
10							
PROJECT INFORMATION				SAMPLE RECEIPT			RELINQUISHED BY (CLIENT)
Client:	Total No. of Containers	1	(Signature) <u>Dennis Perry</u>	(Time) <u>10:30AM</u>	(Printed Name) <u>Dennis Perry</u>	(Date) <u>12/10/12</u>	(Signature)
PO No.:	Received Good Condition?	Y	(Signature) <u>Dennis Perry</u>	(Time) <u>10:30AM</u>	(Printed Name) <u>Dennis Perry</u>	(Date) <u>12/10/12</u>	(Signature)
Shipped Via:	Matches Test Schedule?	Y	(Signature) <u>Washington Beef LLC</u>	(Time)	(Printed Name) <u>Washington Beef LLC</u>	(Date)	(Signature)
RECEIVED BY (COURIER)				RECEIVED BY (LABORATORY)			Receipt Temperature (°C)
(Printed Name)	(Date)	(Log In #)	(Signature) <u>Eric Tollefson</u>	(Time) <u>14:00</u>	(Printed Name) <u>ERIC TOLLEFSON</u>	(Date) <u>12/10/12</u>	12-053
SPECIAL INSTRUCTIONS/COMMENTS:							

Rainier ENVIRONMENTAL

Washington
5013 Pacific Highway East, Suite 20 Fife,
WA 98424
Phone 253.922.8898

Date 12/12/12 Page 1 of 1

Sample Collection By:

Report to:
Company Washington Beef LLC
Address 201 Elmwood Rd
City/State/Zip Tumwater, WA 98501
Contact Sherry Beiers Edel
Phone 360-432-6534
Email Sherry.Beiers@ABFuelsUSA.com

Invoice To:
Company _____
Address _____
City/State/Zip _____
Contact Spire
Phone _____
Email _____

ANALYSES REQUIRED

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER	NO. OF CONTAINERS	COMMENTS
Final Outfall 12/12/12	7:40 AM	waste water	laboratory	1	1	
2						
3						
4						
5						
6						
7						
8						
9						
10						

W ET

Receipt Temperature (°C)

10

PROJECT INFORMATION

SAMPLE RECEIPT

RELINQUISHED BY (CLIENT)

(Signature)

(Printed Name)

Mario Garsc

(Time)

12:30 PM

(Date)

12-12-12

(Company)

Washington Beef

(Signature)

(Printed Name)

Eric Tollefson

(Time)

9:30

(Date)

10/3/12

(Company)

ERIC TOLLEFSON

(Signature)

(Printed Name)

Eric Tollefson

(Time)

12-054

(Log In #)

SPECIAL INSTRUCTIONS/COMMENTS:

1

RECEIVED BY (COURIER)

(Signature)

(Printed Name)

Erie Tollefson

(Time)

9:30

(Date)

10/3/12

(Company)

ERIC TOLLEFSON

(Signature)

(Printed Name)

Eric Tollefson

(Time)

12-054

(Log In #)



Washington
5013 Pacific Highway East, Suite 20 Fife,
WA 98424
Phone 253.922.8898

Dated 12/14/12 Page 1 of 1

Chain of Custody

Sample Collection By:

Report to:
Company Washington Bees LLC
Address 201 Elmwood Rd
City/State/Zip Tacoma, WA 98448
Contact: Sherry Byers - Eddy
Phone: 509-452-6534
Email: Sherry.Byers@Abundance.com

Invoice To:

Company
Address S. J. Me
City/State/Zip
Contact:
Phone:
Email:

ANALYSES REQUIRED

Receipt Temperature (°C)

W.E.T.

SAMPLE ID	DATE	TIME	MATRIX	CONTAINER TYPE	NO. OF CONTAINERS	COMMENTS	
						(Signature)	(Time)
1	<u>Farm Bureau</u>	<u>12/14/12</u>	<u>7:18 AM</u>	<u>Water</u>	<u>Cubitainer</u>	<u>1</u>	<u>0.5</u>
2							
3							
4							
5							
6							
7							
8							
9							
10							

PROJECT INFORMATION

SAMPLE RECEIPT

RELINQUISHED BY (CLIENT)

(Signature)

(Time)

(Signature)

(Time)